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(54) Chimaeric interleukin 5-receptor/immunoglobulin polypeptides.

(57) The present invention is directed to a DNA sequence comprising two partial DNA sequences, one partial sequence coding for a fragment of the α- and/or the β-chain of the human interleukin-5 receptor which fragment or combination of fragments binds human interleukin-5 and the other partial sequence coding for the constant domains of a heavy- or a light chain of a human immunoglobulin such as IgG, IgA, IgM or IgE or a part thereof, a vector comprising such a DNA sequence, a pro- or eukaryotic host cell transformed with such a vector, a recombinant protein coded for by such a DNA sequence, a process for the preparation of such recombinant protein and a pharmaceutical composition containing such a recombinant protein.

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Interleukin-5 (IL-5 or IL5) is a lymphokine secreted by T cells and mast cells having biological activities on B cells and eosinophils. The activity on B cells seems to be restricted to the murine system. No detectable activity can be found in a panel of human B-cell activation or differentiation assays. [Clutterbuck et al., Eur. J. Immunol. 17, 1743-1750 (1987)].

In murine hematopoiesis, IL-5 is a selectiv signal for the proliferation and differentiation of the eosinophilic lineage [Yamaguchi et al., J. Exp. Med. 167, 43-56 (1988)]. In this respect, IL-5 function shows analogies with colony-stimulating factors for other myeloid lineages. Also, human (h) IL-5 is very potent in the activation of human eosinophils [Lopez et al., J. Exp. Med. 167, 219-224 (1988); Saito et al., Proc. Natl. Acad. Sci USA 85, 2288-2292)].

Interleukin 5 mediates its activity through a cell membrane receptor-complex. This complex has been characterized physicochemically in both the murine and human system. Mouse pre B cell lines depending on IL5 for their growth have been developed from bone marrow and are used for IL5-receptor analysis [Rolink et al., J. Exp. Med. 169, 1693-1701 (1989)]. The human IL5-receptor can be studied on a subclone of the promyelocytic cell line HL60 induced towards eosinophil differentiation [Plaetinck et al., J. Exp. Med. 172, 683-691 (1990)].

Eosinophilic differentiation is initiated using sodium butyrate. Only high affinity (Kd = 30 pM) IL5 binding sites can be found on these cells. However cross-linking studies reveal the presence of two polypeptide chains involved in IL5 binding, with molecular masses closely resembling the murine IL5R-α- and -β chains.

A soluble human IL5Rα-chain (shIL5Rα) could be used as an IL-5 antagonist in chronic asthma or other disease states with demonstrated eosinophilia. In addition the shIL5Rα or the α-chain itself or the whole high affinity receptor, consisting of the α-chain and the β-chain [Tavernier et al., Cell 66, in press (1991)] could be used as a tool for screening for IL-5 antagonists.

It is therefore an object of the present invention to provide DNA sequences which comprise a combination of two partial DNA sequences, with one of the partial sequences coding for a fragment of the α - and/or the β-chain of the hIL5R which fragment or combination of fragments binds hIL5 whereby a fragment of the α-chain (shIL5Rα) and especially such a fragment with the whole or a part of the sequence as shown in Figure 1 is preferred and the other partial sequence coding for part or all constant domains of human immunoglobulin heavy- or light chains wherein heavy chains, especially all domains except the first domain of the constant domain of human immunoglobulins such as IgG, IgA, IgM or IgE and specifically IgG, e.g. IgG1 and IgG3 are preferred. It is furthermore understood that a DNA sequence coding for a fragment of the α-chain of the hIL5R which fragment binds hIL5 comprises also DNA sequences which hybridize under stringent hybridization conditions to a DNA sequence as shown in Figure 1 coding for a protein which binds hIL5. A man skilled in the art will easily be able to define such stringent hybridization conditions based on the DNA-sequence shown in Figure 1 and according to standard knowledge in the state of the art and disclosed e.g. in Sambrook et al.. It is furthermore an object of the present invention to provide vectors comprising such DNA sequences, especially such vectors capable of expression in eukaryotic host cells and pro- or eukaryotic host cells transformed with such vectors. Finally it is an object of the present invention to provide a process for the preparation of the recombinant proteins coded for by such DNA-sequences which process comprises cultivating a transformed host as mentioned before in a suitable medium and isolating the recombinant protein.

The present invention is also concerned with the recombinant chimaeric polypeptides coded by such DNA sequences, especially for the treatment of illnesses, e.g. chronic asthma. Of course, there are thereby also included such proteins in the amino acid sequences of which amino acids are deleted or exchanged, so that the activity of the proteins is not significantly changed. Amino acid exchanges in proteins and peptides which do not generally alter the activity of such molecules are known in the state of the art and are described, for example, by H. Neurath and R.L. Hill in "The Proteins" (Academic Press, New York, 1979, see especially Figure 6, page 14). The most commonly occurring exchanges are: Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, Asp/Gly as well as these in reverse.

Such chimaeric polypeptides could have increased half-life in vivo. Increased half-life in vivo has been shown e.g., for chimaeric polypeptides consisting of the first two domains or parts thereof of the human CD4-molecule and different domains of the constant regions of the heavy chain or the light chain of a mammalian immunoglobulin (see Traunecker et al., Nature 331, 84-86 [1988] and European Patent Application, Publication No. 394 827).

It is furthermore understood that for the purpose of the present invention the chimaeric polypeptides can be in a dimeric form, namely consisting either of two subunits whereby each subunit comprises a fragment of the α-chain of the IL5R which still binds hIL5 or of two subunits whereby one of the two

subunits comprises a fragment of the β chain of the IL5R so that the dimeric polypeptide binds hIL5.

The cloning of a DNA sequence coding for the α-chain of the hIL5R can be achiev d in the following manner. Murine cell lines which contain the murine IL-5-receptor (mIL5R) in membrane-bound form, can b cultivated according to methods known in the art or as specifically described in e.g. in Example 2. Such cells can then be harvested by centrifugation, lysed and a membrane extract can be prepared by using a suitable detergent, e.g. Triton-X-100. For the isolation of the α-chain of mIL5R, the membrane extract, cleared by centrifugation, can be passed over an immuneaffinity matrix. The corresponding antibodies for such an immunematrix namely the ones to the α-chain of the mIL5R can be prepared and coupled to an appropriate matrix by methods well known in the art or as specifically described e.g. in Examples 1-3. The α-chain of the mIL5-R can be further purified by sodium dodecylsulfate polyacrylamide gelelectrophoresis (SDS-PAGE) and blotted to an appropriate matrix.

The thus-purified murine IL-5-receptor chain can be characterized by methods of peptide chemistry which are known in the state of the art, such as, for example, N-terminal amino acid sequencing or enzymatic as well as chemical peptide cleavage. Fragments obtained by enzymatic or chemical cleavage can be separated according to usual methods such as, for example, HPLC and can themselves be subjected to further N-terminal sequencing.

Starting from the so-obtained amino acid sequence information oligonucleotides can be produced according to methods known in the state of the art [see e.g. Sambrook et al., s.a.] taking into consideration the degeneration of the genetic code.

cDNA or genomic DNA libraries can be produced according to methods known in the art [Sambrook et al., "Molecular Cloning", 2nd. ed., Cold Spring Harobor Laboratory Press (1989)], whereby cDNA libraries on the basis of an mRNA-preparation from cell lines expressing with or without induction murine or human IL5R, e.g. as specifically described in Example 4, are preferred. Such libraries can then be screened by oligonucleotides [Sambrook et al., s.a.]. Once a specific clone has been identified in such a manner the phage harboring the desired DNA sequence of the invention can be isolated [Sambrook et al., s.a.] and the corresponding inserts characterized by restriction enzyme cleavage pattern analysis or sequencing according to standard procedures (Sambrook et al., s.a.). It is understood that also DNA sequences hybridizing under stringent hybridization conditions (see e.g. Sambrook et al.; s.a.) to those of the present invention and coding for proteins which bind IL5 can be employed for the purpose of the present invention. Such DNA sequences can be prepared e.g. by mutagenesis methods known in the art (see e.g. Sambrook et al.) starting from the corresponding non-mutated DNA sequences. Furthermore the polymerase chain reaction (PCR) can be used for the preparation of DNA sequences of the present invention as described e.g. in detail in examples 12 and 13.

On the basis of the thus-determined sequences and of the already known sequences for certain receptors, those partial sequences which code for a soluble receptor subunit can be determined and cut out from the complete sequence using known methods [Sambrook et al., s.a., see also Maliszewski and Fanslow, Tibtech., 8, 324-329 (1990)] in case that a specific insert cDNA of a clone of the present invention does not already code for such a shIL5Rα as this is for example the case for cDNA clone "λgt11-hIL5Rα12".

The complete sequence or such partial sequences can then be integrated using known methods into vectors described in the state of the art for their amplification and/or expression in prokaryotes [Sambrook et al., s.a.]. Suitable prokaryotic host organisms are, for example, gram-negative and gram-positive bacteria such as, for example, E. coli strains such as E. coli HB 101 [ATCC No. 33 694] or E. coli W3110 [ATCC No. 27 325] and E.coli MC1061 [Casadabam and Cohen, J. Mol. Biol. 138, 179-207 (1980)], the latter two already harboring plasmid "p3" (Sambrook et al., s.a.) in case that pCDM8-type vectors like πVX or pshIL5Rα (see Example 9) will be amplified or B. subtilis strains.

Furthermore such sequences can be integrated using known methods into suitable vectors for expression in eukaryotic host cells, such as, for example, yeast, insect cells and mammalian cells.

A typical expression vector for mammalian cells contains an efficient promoter element in order to produce a good transcription rate, the DNA sequence to be expressed and signals for an efficient termination and polyadenylation of the transcript. Additional elements which can be used are "enhancers" which lead to again intensified transcription and sequences which e.g. can bring about a longer half life of the mRNA. For the expression of nucleic acid sequences in which the endogenous sequence fragment coding for a signal peptide is missing, there can be used vectors which contain such suitable sequences which code for signal peptides of other known proteins. See, for example, the vector pLJ268 described by Cullen, B.R. in Cell 46, 973-982 (1986) as well as Sharma, S. et al. in "Current Communications in Molecular Biology", edt. by Gething, M.J., Cold Spring Harbor Lab. (1985), pages 73-78.

Most of these vectors which ar used for a transient expression of a particular DNA sequence in mammalian cells contain the replication origin of the SV40 virus. In cells which express the T-antigen of the virus (e.g. COS cells), these vectors are reproduced abundantly. A transient expression as described .g. in Example 10 is, however, not limited to COS cells. In principle any transfectable mammalian cell line can be used for this purpose. Signals which can bring about a strong transcription are e.g. the early and late promoters of SV40, the promoter and enhancer of the "major immediate-early" gene of HCMV (human cytomegalovirus), the LTR's ("long terminal repeats") of retroviruses such as, for example, RSV, HIV and MMTV. There can, however, also be used signals of cellular genes such as e.g. the promoters of the actin and collagenase genes.

Alternatively, however, stable cell lines which have the specific DNA sequence integrated into the genome (chromosome) can also be obtained. For this, the DNA sequence is cotransfected together with a selectable marker, e.g. neomycin, hygromycin, dihydrofolate reductase (dhfr) or hypoxanthin guanine phosphoribosyl transferase (hgpt). The DNA sequence stably incorporated in the chromosome can also be amplified abundantly. A suitable selection marker for this is, for example, dihydrofolate reductase (dhfr). Mammalian cells (e.g. CHO cells), which contain no intact dhfr gene, are thereby incubated with increasing amounts of methotrexate after transfection has been effected. In this manner cell lines which contain more than a thousand copies of the desired DNA sequence can be obtained.

Mammalian cells which can be used for expression are e.g. cells of the human cell lines Hela [ATCC CCL2] and 293 [ATCC CRL 1573] as well as 3T3 [ATCC CCL 163] and L cells, e.g. [ATCC CCL 149], (CHO) cells [ATCC CCL 61], BHK [ATCC CCL 10] cells as well as the CV 1 [ATCC CCL 70] and the COS cell lines [ATCC CRL 1650, CRL 1651].

Suitable expression vectors include, for example, vectors such as pBC12MI [ATCC 67 109], pSV2dhfr [ATCC 37 146], pSVL [Pharmacia, Uppsala, Sweden], pRSVcat [ATCC 37 152], pMSG [Pharmacia, Uppsala, Sweden] and pCDM8 type plasmids like e.g. pshIL5Rα [see Example 7] which has been deposited transformed in E.coli MC1061 (harboring plasmid p3) under the conditions of the Budapest Treaty for patent purposes at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, Federal Republic of Germany on April 17, 1991 under accession number DSM 6479 The plasmid pshIL5Rα can be isolated from the deposited transformed E.coli as known in the art and described e.g. in detail in Example 9. For the expression of the chimaeric polypeptides of the present invention, pSV2-derived vectors [see for example German, C. in "DNA Cloning" Vol. II., edt. by Glover, D.M., IRL Press, Oxford, 1985] like pCD4-Hμ (DSM 5315), pCD4-Hγ1 (DSM 5314) and pCD4-Hγ3 (DSM 5523) which have been deposited at the Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, FRG, and which are described in detail in European Patent Application No. 90107393.2, Publication No. 394 827 can be used. The specification of this European Patent Application contains also data with respect to the further use of these vectors for the expression of chimaeric proteins and for the construction of vectors for the expression of such chimaeric proteins with other immunoglobulin fragments. For the purpose of the present invention the CD4 coding part in these vectors has to be replaced by a DNA sequence coding for a fragment of the α-and/or β-chain of the hIL5R which still binds hIL5 by methods known in the art and described e.g. in Sambrook et al. (s.a.) and if desirable in such vectors obtained, the specific immunoglobulin coding part can be replaced by a DNA sequence coding for the desired immunoglobulin part. Preferred vectors for the expression of the chimaeric polypeptides of the present invention are pCDM8 type vectors like e.g. pshIL5Rα for the expression of fragments of the α-chain of the IL5R containing chimaeric polypeptides (see examples 12 and 13). As already described above sources for DNA sequences coding for constant domains of human immunoglobulins are known in the state of the art and disclosed for example also in EP 394 827 or are described e.g. by Ellison et al. [Nucl. Acid Res. 10, 4071-4079 (1982)] or Huck et al. [Nucl. Acid Res. 14, 1779-1789 (1986)] in case of such constant domains of the IgG1 or IgG3, respectively.

The manner in which these cells are transfected depends on the chosen expression system and vector system. An overview of these methods is to be found e.g. in Pollard et al., "DNA Transformation of Mammalian Cells" in "Methods in Molecular Biology" [Nucleic Acids Vol. 2, 1984, Walker, J.M., ed, Humana, Clifton, New Jersey]. Further methods are to be found in Chen and Okayama ["High-Efficiency Transformation of Mammalian Cells by Plasmid DNA", Molecular and Cell Biology 7, 2745-2752, 1987] and in Felgner [Felgner et al., "Lipofectin: A highly efficient, lipid-mediated DNA-transfection procedure", Proc. Nat. Acad. Sci. USA 84, 7413-7417, 1989]. Eukaryotic host cells transfected with a suitable plasmid (vector) of the invention, as well as the plasmids used for their transfection and expression of the corresponding recombinant protein, are also an object of the present invention.

The baculovirus expression system, which has already been used successfully for the expression of a series of proteins (for an overview see Luckow and Summers, Bio/Technology 6, 47-55, 1988), can be used

for the expression in insect cells. Recombinant proteins can be produced in authentic form or as fusion proteins. The thus-produced proteins can also be modified such as, for example, glycosylated (Smith et al., Proc. Nat. Acad. Sci. USA 82, 8404-8408, 1987). For the production of a recombinant baculovirus which expresses the desired protein there is used a so-called "transfer vector". Under this there is to be understood a plasmid which contains the heterologous DNA sequence under th control of a strong promoter, e.g. that of the polyhedron gene, whereby this is surrounded on both sides by viral sequences. The transfer vector is then transfected into the insect cells together with DNA of the wild type baculovirus. The recombinant viruses which result in the cells by homologous recombination can then be identified and isolated according to known methods. An overview of the baculovirus expression system and the methods used therein is to be found in Luckow and Summers, "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures", Texas Agricultural Experimental Station, Texas A & M University, Bulletin No. 1555, 2nd edition, 1988. It is understood that for the practice of the present invention when using the baculovirus expression system DNA-sequences coding for the immunoglobulin part have to be in the form of a cDNA.

The chimaeric polypeptides of the present invention can then be purified from the cell mass or the culture supernatants according to methods of protein chemistry which are known in the state of the art, such as, for example, precipitation e.g. with ammonium sulfate, dialysis, ultrafiltration, gelfiltration, ion-exchange chromatography, SDS-PAGE, isoelectric focusing, affinity chromatography like immunoaffinity chromatography, HPLC in normal or reverse phase systems or the like.

The chimaeric polypeptides of the present invention as well as their physiologically compatible salts, which can be manufactured according to methods which are known in the state of the art, can also be used for the treatment of illnesses in which IL-5 is involved in their course and/or the production of corresponding pharmaceutical preparations. For this purpose, one or more of the said compounds, where desired or required in combination with other pharmaceutically active substances, can be processed in a known manner with the usually used solid or liquid carrier materials. The dosage of such preparations can be effected having regard to the usual criteria in analogy to already used preparations of similar activity and structure. Such pharmaceutical preparations and the use of the compounds of the present invention for therapeutical purposes are also an object of the present invention.

After the invention has been described in general hereinbefore, the following Figures and Examples are intended to illustrate details of the invention, without thereby limiting it in any manner.

Figure 1

Nucleic acid sequence of shIL5Rα and deduced amino acid sequence whereby the corresponding amino acid sequence of the mIL5Rα is indicated below by showing only such amino acids which are different from the ones of the human sequence. Sequences are represented by standard abbreviations for nucleotides and amino acids.

Figure 2

Figure 2 gives the results of competition binding assay employing shIL5Rα. For details see Example 11.

Example 1

Production of monoclonal antibodies against the murine IL5R

Immunization was carried out basically as described by A. Rolink et al. (s.a.). Briefly: at day 0, $2X10^7$ B13 cells [Rolink et al., s.a.] were washed with phosphate buffered saline (PBS-A), mixed with complete Freund's adjuvant (CFA) and injected into the hind footpath of Wistar rats. This was repeated without Freund's adjuvant (FA) on day 5 and 7. On day 8, regional lymph nodes were removed and a cell suspension was prepared. These cells were fused using PEG 1500 (Boehringer) with Sp2/0-Ag14 cells [ATCC CRL 1581] at a ratio of 5:1,5. Cells were plated in microtiterplates in the presence of 500 pg/ml of recombinant hIL-6 [Haegemann et al., Europ. J. Biochem. 159, 625-632 (1986)]. The next day, the same volume of medium containing a 2x conc. of aminopterin was added for selection of hybrid cells. Cells were refed at day 8 with medium without aminopterin. Hybridomas were selected on the ability of their supernatant to inhibit the mIL5 [Tavernier, J. et al., DNA 8, 491-501 (1989)] or an mouse interleukin-3 (mIL3) driven proliferation of B13 cells (measured by a ^{3}Hdeoxy-cytidin incorporation assay as known in the art). Conditioned medium from WEHI-3 cells (ATCC No. TIB68) was used as a source of mIL3. Supernatants

demonstrating inhibiting activity were retested in a competition-binding assay with radiolabeled [according to methods known in the art] mIL5 or "R52" [a monoclonal antibody recognizing only the β-chain of the IL-5-R (Rolink et al. s.a.)] on B13 cells. Monoclonal antibodies directed only to the α-chain of the mIL-5-R were identified on their ability to inhibit almost completely mIL5 binding and by immunprecipitation of the corr sponding mIL-5-R chain. Selected hybridomas were recloned by th limiting dilution method.

Example 2

Immunoaffinity purification of the mIL5R-β-chain

B13 cells were grown in large spinner flasks in Iscove's modified Dulbecco's medium (Gibco Laboratories, grand Island N.Y., USA) containing 5% fetal calf serum, 2mM L-glutamine, 50µg/ml gentamycin, and 100 units/ml recombinant mouse IL-5, to a density of 2 x 10^6 cells/ml. Cells from 10 l cultures were concentrated by centrifugation, washed with PBS and lysed in 200ml PBS containing 1% Triton-X-100 and a cocktail of protease inhibitors (1mM PMSF, 10mM benzamidine.HCl, 100 U/ml aprotinin). After 10 min on ice, the lysate was centrifuged for 10 min at 1000 x g and cleared by ultracentrifugation (100.000 x g) for 90 min at 4°C. The supernatant was diluted with NaCl to a final concentration of 0.5 M, and used for purification. "R52" was covalently bound to protein G-Sepharose 4 Fast Flow (Pharmacia, LKB Biotechnology AB, Uppsala, Sweden) according to Schneider et al.[J. Biol. Chem. 257, 10766 (1982)], at a concentration of 5 mg/ml gel. Two hundred ml lysate of B13 cells was passed at 4C° over 2 ml protein G-Sepharose 4 Fast Flow followed by 2ml R52-linked protein G-Sepharose 4 Fast Flow both packed in a 1cm diameter column. The flow through was then reloaded on both columns. The gel was washed extensively (100ml) with a buffer containing 50mM Tris-HCl (pH8.2), 1mM EDTA, 0.5M NaCl, 0.5% NP40, followed by 10 ml 0.1% (NP40). Next, the retained proteins were eluted in 4ml 50mM diethylamine (pH11) containing 0.1% Nonidet P40 (NP40), neutralized by addition of 1M NaH_2PO_4 and concentrated by lyophilization. The purity was assessed by SDS-PAGE and Coomassie staining of 2.5% of the eluate.

Example 3

Immunoaffinity purification of the murine IL5R α-chain

B13 cell lysates from 2 x 10^{10} cells (run through fractions of the "R52"-immunoaffinity column used to purify the β-chain doublet according to Example 2) were mixed overnight by 4°C with 2ml hydrazide avidgel AX (Bioprobe Int. Inc.) armed with 10 mg mAbs recognizing the mIL5R α-chain. The gel was then poored into a column, and after extensive washing (50mM Tris.HCl, pH 8.2, 1mM EDTA, 0.5M NaCl, 0.5% NP40; followed by 0.1% NP40 in H_2O) elution was performed using 50mM diethylamine, pH11, 0.01% NP40. Selected fractions were immediately lyophilized and resuspended in 2x Laemmli buffer, in the presence of β-mercaptoethanol. Samples were run through a 1.5 mm 10% PAGE-SDS gel. The gel was fixed in 10% HAc, 30% methanol and stained with Coomassie Brilliant Blue. Slices containing the 60 kDa mIL5R α-chain were treated with SDS buffer, sliced further and electrophoresed in a new PAGE-SDS gel.

After transfer to an Immobilon-P membrane (Millipore Corp.), and staining with amido black, the 60 kDa band was excised and in situ digested with trypsin. Peptides were separated on a C4-reversed-phase column and subjected to sequence analysis using a 470A-type gas-phase sequenator equipped with an on-line 120A-type PTH-amino acid analyser (Applied Biosystems Inc., Foster City, CA). Amino acid sequences (standard abbreviations of amino acids) and the sequences of corresponding sets of oligonucleotide probes, synthesized according to methods known in the art, are shown below:

peptide 1

1	2	3	4	5	6	7	8	9	10	11	12
W	G	E	W	S	Q	P	I	Y	V	G	K

oligonucleotide-set 1 : 32-mers

```
                T
5' CC IAC GTA AAT IGG CTG IGA CCA CTC ICC CCA 3'
          A   G       T           T

                T
5' CC IAC GTA AAT IGG CTG ACT CCA CTC ICC CCA 3'
          A   G       T   G       T
```

peptide 2

```
1 2 3 4 5 6 7 8
H V D L E Y H V
```

oligonucleotide-set 2 : 23-mers

```
5' AC ATG ATA TTC TAA ATC IAC ATG 3'
     G   G   C   C   G       G

5' AC ATG ATA TTC IAG ATC IAC ATG 3'
     G   G   C       G       G
```

Example 4

Construction of unidirectional λGT11 cDNA libraries

1. Murine pre-B cell B13 cDNA library

mRNA was extracted from B13 cells using the "fast-track" mRNA isolation system (Invitrogen Corp.). Using this protocol, poly(A)$^+$ mRNA was directly isolated from cell lysates using oligo(dT) cellulose; yields were around 50 µg per 10^8 cells. 5 mg poly(A)$^+$ mRNA was reverse transcribed using an oligo dT-Not1 primer-adaptor (5'-AATTCGCGGCCGC$(T)_{15}$-3', Promega Corp.) and cloned Moloney Murine Leukemia Virus RNaseH$^-$ Reverse Transcriptase (BRL Life Technologies, Inc.). EcoR1 linkered double stranded cDNA was made using described procedures (Sambrook et al., s.a.). Not1 cleavage was used to generate a unique 3' sticky-end, and cDNAs were size selected (>1.000 bp) on a 1% agarose gel. After elution using the "gene clean" protocol (BIO 101 Inc.), cDNAs were ligated into the EcoR1-Not1 arms of the λgt11 Sfi-Not vector (Promega Corp.). After in vitro packaging around 40 x 10^6 recombinant phages were obtained.

2. Human. HL60 clone (butyrate induced) cDNA library

Prior to mRNA purification, butyrate induced HL60 clone 15 cells [Fischkoff, Leukemia Res. 12, 679-686 (1988); Plaetinck et al., J. Exp. Med. 172, 683-691 (1990); HL60: ATCC-No. CCL 240] were checked for proper ^{125}I-hIL5 binding (around 2000 binding sites per cell). The same protocols as for 4.1 were used, and a comparable yield of recombinant phage was obtained.

Example 5

Screening of murine and human cDNA libraries

2 sets of oligonucleotide probes "Oligonucleotide 1" and "Oligonucleotide 2", see Example 3 were used for screening under different hybridization conditions (see below), dependent on the type of probe used by methods known in the art (Sambrook et al., s.a.). Results are presented in the scheme below:

1. 2 cDNA clones (λgt11-mIL5Rα2,3) were selected from part of the murine cDNA library ($1.2x10^6$ plaques were screened), on the basis of hybridization with both sets of oligonucleotide probes. For that purpose, plaque lifts were prepared as described using Biodyne A transfer membranes (Pall), (Sambrook et al., s.a.). Oligonucleotide 1 was radioactively labeled by kinasing (Sambrook et al., s.a.) and was hybridized under "intermediate stringency" hybridization conditions (see below). Oligonucleotide 2 was radioactively labeled by kinasing (Sambrook et al., s.a.) and was hybridized under "low stringency" hybridization conditions (see below).
2. 1 cDNA clone (λgt11-hIL5Rα8) was selected from part of the human cDNA library (2.4×10^6 plaques were screened), on basis of hybridization with both "oligonucleotide 1" and the cDNA insert derived by methods known in the art from the murine λgt11mIL5Rα2.

   Oligonucleotide 1 was radioactively labeled by kinasing (Sambrook et al., s.a.) and was hybridized under "low stringency" hybridization conditions. The cDNA insert form λgt11mIL5Rα2 was radioactively labeled by random labeling (Sambrook et al., s.a.) and was hybridzed under "intermediate stringency" hybridization conditions.
3. 5 additional cDNA clones (λgt11-hIL5Rα11→15) were selected from half of the human cDNA library screened in 2. using the mIL5Rα2 cDNA probe. Hybridization was under "intermediate stringency" conditions.
4. 35 additional cDNA clones (λgt11-hIL5Rα16→51) were selected from the other half of the human cDNA library screened in 2. using the hIL5Rα8-cDNA probe. Hybridization was under "high stringency" conditions (see below).

Hybridization conditions

L) "low stringency" hybridization conditions:

- prehdybridization: 5 x SSC (citrate buffered salt solution known in the art, see e.g. Sambrook et al., s.a.), 5x Denhardt's, 0.1% SDS, 0.05% sodium pyrophosphate, 100 µg/ml sonicated salmon sperm DNA; overnight at 42 °C.
- hybridization: prehybridization buffer was replaced by the same buffer but including the radioactively labeled probe.
- washes: 4 consecutive washes (around 30 min. each) with 2x SSC, 0.1% SDS at 37 °C:

I) "intermediate stringency" hybridization conditions:

- prehybridization: 20% formamide, 5x SSC, 5x Denhardt's, 5mM EDTA, 25mM sodium phosphate (pH 6.5), 0.05% sodium pyrophosphate, 100 µg/ml sonicated salmon sperm DNA; overnight at 42 °C.
- hybridization: prehybridization buffer was replaced by the same buffer but including the radioactively labeled probe.
- washes: 4 consecutive washes (around 30 min. each) with 2x SSC, 0.1% SDS at 37 °C.

H) "high stringency" hybridization conditions:

- prehybridization: 6x SSC, 5x Denhardt's, 0.5% SDS, 100 $\mu g.ml^{-1}$, sonicated salmon sperm DNA, overnight at 68 °C.
- hybridization: 6x SSC, 5x Denhardt's, 0.5% SDS, 5mM EDTA, 100$\mu g.ml^{-1}$ sonicated salmon sperm DNA including the radioactively labeled probe.
- washes: the following consecutive washes (around 30 min. each) were performed:
- 2x SSC, 0.1% SDS at room temperature (twice).
- 0.1x SSC, 0.1% SDS at 68 °C (twice).

Example 6

Sequencing

All cDNAs were subcloned in pGEM7zf type vectors (Promega Corp.), and Exo III deletion mutants have been generated. Sequencing was performed using a protocol based on the Sanger procedure and involving Taq polymerase and single stranded DNA on an automated 370A DNA Sequencer.

Example 7

Construction of plasmid "pshIL5Rα"

Plasmid constructions were carried out as described in the following paragraphs. In case that no specific references or details of preparation are given standard methodology according to Sambrook et al. (1989), Molecular Cloning. A Laboratory Manual (2nd edn). Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press, was used.

The insert from phage λgt11-hIL5Rα12 (see Example 5) was excised using EcoR1 and Not1 restriction enzymes. Both sticky ends were filled in using E.coli DNA polymerase 1 Klenow fragment in the presence of all four deoxynucleotide triphosphates, and non-palindromic BstX1 linkers were added using T4 DNA ligase. The sequence of these linkers is as follows:

5' CTTTAGAGCACA 3'
3' GAAATCTC 5'.

In a next step, the modified insert was ligated into plasmid pCDM8 [Seed and Aruffo, Proc. Natl. Acad. Sci. USA, 84, 3365 (1987); Aruffo and Seed, Proc. Natl. Acad. Sci. USA, 84, 8573 (1987); Seed, Nature, 329, 840 (1987)] and the construct with the appropriate orientation versus the CMV-promoter was chosen for further analysis.

Example 8

Transformation of E.coli MC1061(p3)

Transformation of E.coli MC1061 (p3) with the plasmid pshIL5Rα of Example 7 was achieved by the electroporation procedure. A Gene Pulser from Bio-Rad (Richmond, CA, USA) with the following settings: 25 µF, 2.5 kV and 200 Ohms was used according to the instructions of the manufacturer.

Example 9

Isolation of Plasmid DNA

Plasmid DNA from transformed E.coli MC1061 as described in Example 8 was prepared using a standard procedure [Birnboim and Doly, Nucl. Acids Res. 7, 1513 (1979); Sambrook et al., 1989, s.a.] based upon alkali lysis of the cells, followed by a cesium-chloride ultracentrifugation step. In this way plasmid pshIL5Rα was separated from plasmid p3. The insert coding for shIL5Rα was cut out of pshIL5Rα and sequenced as described in Example 6. The complete nucleic acid sequence and the deduced amino acid sequence of the shIL5Rα are shown in Figure 1.

Example 10

Expression of shIL5Rα in COS-1 cells

COS-1 cells were transfected using the DEAE-Dextran protocol as described in Sambrook et al., 1989, s.a.. Briefly, subconfluent COS-1 cells were harvested by trypsinization and replated at 2.3 x 10^4 cells/cm^2 24 hours prior to transfection. The monolayers were washed twice with minimal essential medium (MEM)-Hep s pH 7.2 and incubated for 30 minutes with the transfection mixture [10 µg pshIL5Rα isolated as

described in Example 9/ 0.5 mg DEAE-dextran (M_r = 2 x 10^6; Pharmacia, Uppsala, Sweden)/ml MEM-Hepes, pH 7.2]. Next the cells were supplemented with 8 volumes prewarmed Dulbecco's modified Eagles medium (DMEM) containing 10% foetal calf serum (FCS) and 100 µM chloroquine diphosphate, and incubated for 4 hours at 37°C. Thereafter the medium was removed by aspiration and the monolayers were washed once with DMEM and incubated for 3 days in DMEM + 10% FCS.

Example 11

Characterization of shIL5Rα

Supernatant of COS-1 cells transfected with plasmid pshIL5Rα prepared as described in Example 10 was tested for the presence of secreted shIL5Rα in a competition binding assay as follows: COS-1 cells transfected as described in Example 10 with a plasmid comprising a cDNA coding for mIL5Rα (for amino acid sequence see Figure 1), obtained from a clone as described in Example 5 and constructed as described in Example 7, were detached by treatment with phosphate buffered saline (PBS) containing 0.5 mM EDTA and 0.02% sodium azide for 30 minutes at 37°C, resuspended at 1.5 x 10^5 cells per 0.3 ml binding medium (DMEM + 10% FCS + 0.02% sodium azide) and incubated with 0.8 nM ^{125}I-mIL5 at 4°C for 1 hour in the absence (Figure 2; "-", total binding) or presence (Figure 2; "+cold", non-specific binding) of 100- fold excess unlabeled mIL5. Supernatant of COS-1 cells (80% of binding medium) transfected with pshIL5Rα (Figure 2, "shIL5Rα") was tested for its capacity to inhibit the binding of ^{125}I-mIL5. Binding was also carried out in the presence of 80% supernatant of untransfected COS-1 cells (Figure 2, "control"). To separate cell membrane bound ^{125}I-mIL5 from free radioactivity COS-1 cells were sedimented through a phthalate oil cushion and individual pellets were counted in a gamma counter as described [Plaetinck et al., J. Exp. Med. 172, 683-691 (1990)].

Example 12

Construction of a chimaeric human IL5Rα-IgG1 molecule

As a first step, a polymerase chain reaction (PCR) was performed using plasmid pshIL5Rα as a template and using the following primers:

5'-CATAGACACGACAGACACGG, located in the 5' untranslated region of the hIL5Rα gene (position 104→123) and

5'-TACTGCAGATCCGCCTCTTGAGAACCCCACAT, a primer which matches the last 17 residues of the coding region of the hIL5Rα soluble form, with the addition of 15 residues coding for a Gly-Gly-Ser-Ala "linker" region, and a Pst1 recognition site. The PCR was performed using Vent Polymerase, under conditions as described by the manufacturer (New England BioLabs Inc., Beverly, MA, USA).

After phenol extraction and ethanol precipitation, the PCR product was resuspended in an appropriate buffer, and was kinased by T4 kinase and blunted by Klenow Polymerase by methods described.

To the blunt ended PCR fragment, Bst X1 recognition sites were added, by ligation of 2 synthetic non-palindromic oligonucleotides with the sequence

5'-CTTTAGAGCACA

and

3'-GAAATCTC.

The resulting fragment was then ligated into Bst X1-opened pCDM8 vector.

The resulting plasmid containing the fragment in a sense orientation relative to the CMV promotor in pCDM8 was opened by Not 1 cleavage, followed by a partial Pst1 restriction digestion. A Pst1-Eag1 restriction fragment was purified from the pBRHIG1 plasmid vector (Ellison et al. s.a.), and ligated into the plasmid vector described above.

Please note that the Eag1 and Not1 restriction enzymes generate the same sticky ends, but that fusion of both causes the loss of the Not1 recognition site, but not of the Eag1 recognition site.

Hence, to favor the desired recombinant construct, a Not1 counterselection was performed.

Example 13

Construction of a chimaeric human IL5Rα-IgG3 molecule

The same protocol as description in Example 12 was used with the following exceptions:
The PCR 5' linker was:

Met
5'-AAGCTT GGATCCATGATCATCGTGGCGCAT
Hind3 BamH1

which creates two extra restriction sites as indicated 5' to the nucleotides which match with the first 6 amino acids of hIL5Rα.

As PCR 3' linker the following nucleotide was used:
5'-GAGCTCACCGGATCCGCCTCTTGAGAACCCCACAT.

In addition a partial Sac1 digest was used instead of a Pst1 digest and pATHIG3(2) (Huck et al. s.a.) was used as a source of the immunoglobulin gene part.

Claims

1. A DNA sequence comprising two partial DNA sequences, one partial sequence coding for a fragment of the α- and/or the β-chain of the human interleukin-5 receptor which fragment or combination of fragments binds human interleukin-5 and the other partial sequence coding for the constant domains of a heavy- or a light chain of a human immunoglobulin such as IgG, IgA, IgM or IgE or a part thereof.

2. A DNA sequence according to claim 1, wherein one partial DNA sequence codes for a fragment of the α-chain of the human Interleukin-5 receptor which still binds human Interleukin 5.

3. A vector comprising a DNA sequence as claimed in claim 1 or 2.

4. A vector as claimed in claim 3 capable of directing expression in eukaryotic host cells.

5. A pro- or eukaryotic host cell transformed with a vector as claimed in claim 3 or 4.

6. A recombinant protein coded for by a DNA sequence as claimed in claims 1 or 2.

7. A recombinant protein as claimed in claim 6 for the treatment of illnesses.

8. A process for the preparation of a protein as claimed in claim 6, which process comprises cultivating a transformed host as claimed in claim 5 in a suitable medium and isolating said protein.

9. A pharmaceutical composition which contains one or more compounds according to claim 6, if desired in combination with additional pharmaceutically active substances and/or non-toxic, inert, therapeutically compatible carrier materials.

10. The use of a compound according to claim 6 for the treatment of illnesses, especially chronic asthma.

Claims for the following Contracting States : GR, ES

1. A process for the preparation of a recombinant protein coded for by a DNA sequence comprising two partial DNA sequences, one partial sequence coding for a fragment of the α - and/or the β -chain of the human interleukin-5 receptor which fragment or combination of fragments binds human interleukin-5 and the other partial sequence coding for the constant domains of a heavy- or a light chain of a human immunoglobulin such as IgG, IgA, IgM or IgE or a part thereof which process comprises cultivating a host cell transformed with a vector capable of expressing such a DNA-sequence in a suitable medium and isolating said protein.

2. A process as claimed in claim 1 wherein one of the partial DNA sequences codes for a fragment of the α-chain of the human interleukin-5 receptor which still binds human Interleukin 5.

3. A process as claimed in claim 1 or 2 whereby said host cell is a pro- or eukaryotic host cell.

4. A process as claimed in claim 3 whereby said eukaryotic host cell is a mammalian host cell.

5. A process as claimed in claim 4 whereby said mammalian host cell is a CHO- or COS-cell.

6. A process as claimed in any one of claims 1-5 whereby the expression vector is of the pSV2-, pRSV-, pBC121MI-, pMSG- or pCDM8-type.

7. A process for the preparation of a pharmaceutical composition which process is characterized in that a compound obtained by a process as claimed in any one of claims 1-6 and if desired, additional pharmaceutically active substances are mixed with a non-toxic, inert, therapeutically compatible carrier material and the mixture is brought into a galenical application form.

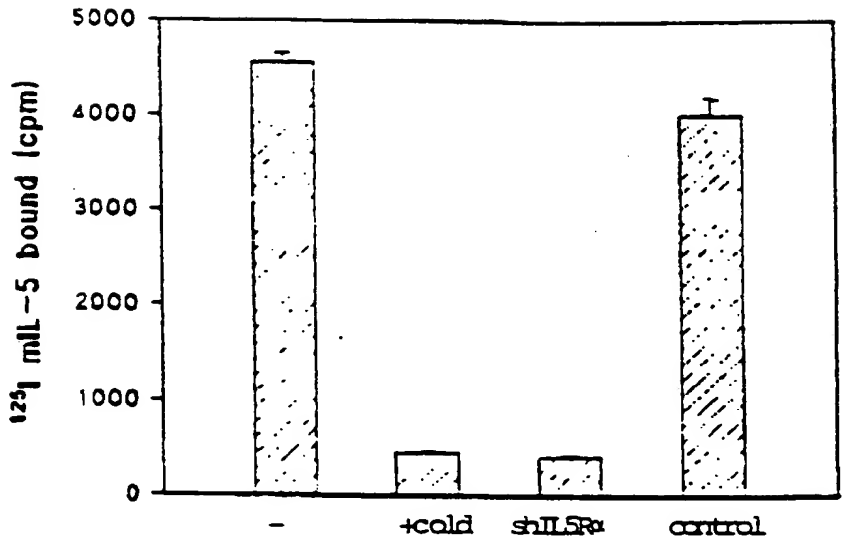
8. A pharmaceutical composition which contains one or more compounds obtained according to a process as claimed in any one of claims 1-6, if desired, in combination with additional pharmaceutically active substances and/or non-toxic, inert, therapeutically compatible carrier materials.

9. The use of a compound prepared according to a process as claimed in any one of claims 1-6 for the preparation of a pharmaceutical composition according to claim 8.

Fig. 1

```
                                                                                                                CCGCTGCTTCTC  12
    ATCGCATGGCCACCGCATTTCTCAGGCCAGGCACATTGAGCATTGGTCCTGTGCCTGACGCTATGCTAGATGCTGGGGT  91
    TGCAGCCACGAGCATAGACACGACAGACACGGTCCTCGCCATCTTCTGTTGATACTGGTCGGAACAAGAGGATCGTCT  170
    GTAGACAGGCTACAGATTGTTTTAGATTGAAGTTTCCTGTCATGTTCACTCATCTTTAAATCCTCATAGTAAAAAGGAT  249

    ATG ATC ATC GTG GCG CAT GTA TTA CTC ATC CTT TTG GGG GCC ACT GAG ATA CTG CAA GCT  309
1   Met Ile Ile Val Ala His Val Leu Leu Ile Leu Leu Gly Ala Thr Glu Ile Leu Gln Ala
    --- --- --- Met Val Pro                         Val         Leu Ala Thr

    GAC TTA CTT CCT GAT GAA AAG ATT TCA CTT CTC CCA CCT GTC AAT TTC ACC ATT AAA GTT  369
21  Asp Leu Leu Pro Asp Glu Lys Ile Ser Leu Leu Pro Pro Val Asn Phe Thr Ile Lys Val
                Asn His Lys     Phe Leu                                         Ala

    ACT GGT TTG GCT CAA GTT CTT TTA CAA TGG AAA CCA AAT CCT GAT CAA GAG CAA AGG AAT  429
41  Thr Gly Leu Ala Gln Val Leu Leu Gln Trp Lys Pro Asn Pro Asp Gln Glu Gln Arg Asn
                                    His     Asp                                 His

    GTT AAT CTA GAA TAT CAA GTG AAA ATA AAC GCT CCA AAA GAA GAT GAC TAT GAA ACC AGA  489
61  Val Asn Leu Glu Tyr Gln Val Lys Ile Asn Ala Pro Lys Glu Asp Asp Tyr Glu Thr Arg
        Asp             His                             Gln         Glu     Asp

    ATC ACT GAA AGC AAA TGT GTA ACC ATC CTC CAC AAA GGC TTT TCA GCA AGT GTG CGG ACC  549
81  Ile Thr Glu Ser Lys Cys Val Thr Ile Leu His Lys Gly Phe Ser Ala Ser Val Arg Thr
    Lys                             Pro         Glu         Ala

    ATC CTG CAG AAC GAC CAC TCA CTA CTG GCC AGC AGC TGG GCT TCT GCT GAA CTT CAT GCC  609
101 Ile Leu Gln Asn Asp His Ser Leu Leu Ala Ser Ser Trp Ala Ser Ala Glu Leu His Ala
            Lys Ser Ser     Thr Thr                     Val                 Lys

    CCA CCA GGG TCT CCT GGA ACC TCA ATT GTG AAT TTA ACT TGC ACC ACA AAC ACT ACA GAA  669
121 Pro Pro Gly Ser Pro Gly Thr Ser Ile Val Asn Leu Thr Cys Thr Thr Asn Thr Thr Glu
                            Val Thr                         His     Val Val

    GAC AAT TAT TCA CGT TTA AGG TCA TAC CAA GTT TCC CTT CAC TGC ACC TGG CTT GTT GGC  729
141 Asp Asn Tyr Ser Arg Leu Arg Ser Tyr Gln Val Ser Leu His Cys Thr Trp Leu Val Gly
    Ser Ser His Thr His         Pro                         Arg

    ACA GAT GCC CCT GAG GAC ACG CAG TAT TTT CTC TAC TAT AGG TAT GGC TCT TGG ACT GAA  789
161 Thr Asp Ala Pro Glu Asp Thr Gln Tyr Phe Leu Tyr Tyr Arg Tyr Gly Ser Trp Thr Glu
    Lys                                                 Phe     Val Leu

    GAA TGC CAA GAA TAC AGC AAA GAC ACA CTG GGG AGA AAT ATC GCA TGC TGG TTT CCC AGG  849
181 Glu Cys Gln Glu Tyr Ser Lys Asp Thr Leu Gly Arg Asn Ile Ala Cys Trp Phe Pro Arg
    Lys                     Arg     Ala     Asn         Thr

    ACT TTT ATC CTC AGC AAA GGG CGT GAC TGG CTT TCG GTG CTT GTT AAC GGC TCC AGC AAG  909
201 Thr Phe Ile Leu Ser Lys Gly Arg Asp Trp Leu Ser Val Leu Val Asn Gly Ser Ser Lys
                Asn             Phe Glu Gln     Ala     His Ile

    CAC TCT GCT ATC AGG CCC TTT GAT CAG CTG TTT GCC CTT CAC GCC ATT GAT CAA ATA AAT  969
221 His Ser Ala Ile Arg Pro Phe Asp Gln Leu Phe Ala Leu His Ala Ile Asp Gln Ile Asn
    Arg Ala         Lys                         Ser Pro Leu                 Val

    CCT CCA CTG AAT GTC ACA GCA GAG ATT GAA GGA ACT CGT CTC TCT ATC CAA TGG GAG AAA  1029
241 Pro Pro Leu Asn Val Thr Ala Glu Ile Glu Gly Thr Arg Leu Ser Ile Gln Trp Glu Lys
            Arg             Val             Ser Asn Ser     Tyr

    CCA GTG TCT GCT TTT CCA ATC CAT TGC TTT GAT TAT GAA GTA AAA ATA CAC AAT ACA AGG  1089
261 Pro Val Ser Ala Phe Pro Ile His Cys Phe Asp Tyr Glu Val Lys Ile His Asn Thr Arg
        Leu                 Asp             Asn         Leu         Tyr         Lys

    AAT GGA TAT TTG CAG ATA GAA AAA TTG ATG ACC AAT GCA TTC ATC TCA ATA ATT GAT GAT  1149
281 Asn Gly Tyr Leu Gln Ile Glu Lys Leu Met Thr Asn Ala Phe Ile Ser Ile Ile Asp Asp
            His Ile     Lys                 Ile Ala     Lys                 Lys

    CTT TCT AAG TAC GAT GTT CAA GTG AGA GCA GCA GTG AGC TCC ATG TGC AGA GAG GCA GGG  1209
301 Leu Ser Lys Tyr Asp Val Gln Val Arg Ala Ala Val Ser Ser Met Cys Arg Glu Ala Gly
    Val     Thr     Ser Ile                                 Pro         Met Pro

    CTC TGG AGT GAG TGG AGC CAA CCT ATT TAT GTG GGG TTC TCA AGA TAA AGGAGATAACATCCA  1272
321 Leu Trp Ser Glu Trp Ser Gln Pro Ile Tyr Val Gly Phe Ser Arg End
    Arg     Gly                                 Lys Glu

    GCTTTCCTGCCCCACACCGTATCTGAAATAAAAACAACAGCAGGGATAGCAGATTAAAAAAAAAAAAAAAAAAAAAAAA  1351
```

Fig. 2


5000
4000
3000
2000
1000
0
^{125}I mIL-5 bound (cpm)
-
+cold
shIL5Rα
control